CLAIMS

1. A method for quantitative and qualitative determination of human papillomavirus (HPV) in a sample comprising the steps of:

i) providing a sample from a patient suspected to be infected by HPV, and optionally extracting the nucleic acid of the sample;

ii) dividing the sample or nucleic acid from the sample in two or more sub-samples;

iii) measuring, simultaneously, the presence and amount of two or more viruses in one of said sub-samples by using a specific primer for amplification of each virus or group of viruses, whereby the primers are designed not to compete during the amplification-reaction, and a specific probe for each virus or group of viruses, whereby the probes are designed not to compete during the amplification-reaction and the detection phase;

iv) determining the amount of said sample by analysis of a nuclear gene in a given amount of another of said sub-samples in a separate amplification reaction; and

v) calculating the amount of each virus or group of viruses per amount of sample from the results of steps iii) and iv).

2. A method according to claim 1, wherein the amplifications in steps iii) and iv) are PCR amplification.

3 A method according to claims 1 or 2, which is a PCR-based fluorescent 5' exonuclease assay.

4. A method according to claims 1, 2 or 3, wherein the viruses in step iii) are chosen from HPV 16, 18, 31, 33, 35, 39, 45, 52, and 58.

5. A method according to any of the above claims, wherein HPV 16, 31, 18, 45 is detected and quantified in one sub-sample and optionally HPV 33, 35, 39, 52, and 58 is detected and quantified in another sub-sample.

6. A method according to any of the above claims, wherein the amount of a human single copy gene is detected and quantified in step iv).

7. A method according to claim 6, wherein the gene is HUMPBGDA, Homo sapiens hydroxymethylbilane synthase gene, accnr M95623.1.

8. A method according to any one of the above claims, which is for detection and diagnose of cervical cancer.

9. A kit for detection and quantification of human papillomavirus, comprising
a) seven amplification primers and three probes for HPV 16, 31, 18, 45 according to Table 1 and 2 of the specification; and optionally
b) eight amplification primers and three probes for HPV 33, 35, 39, 52, and 58, according to Table 1 and 2 of the specification .

10. A kit according to claim 9, further comprising
c) two amplification primers and one probe, according to Table 1 and 2 of the specification, for detection and quantification of the amount of a human single copy gene.

11. A kit according to claim 10, wherein the gene is HUMPBGDA, Homo sapiens hydroxymethylbilane synthase gene, accnr M95623.1.

12. A kit according to any of the claims 9-11, further comprising
d) at least two different fluorophores,

13. A kit according to any of the claims 9-12, comprising
a) seven amplification primers and three probes for HPV 16, 31, 18, 45 according to Table 1 and 2 of the specification;
b) eight amplification primers and three probes for HPV 33, 35, 39, 52, and 58, according to Table 1 and 2 of the specification;
c) two amplification primers and one probe, according to Table 1 and 2 of the specification, for detection and quantification of the amount of a human single copy gene; and
d) three different fluorophores.

14. A kit according to any of the claims 9-13 for detection and diagnose of cervical cancer.

ABSTRACT

The present invention relates to a method and kit for quantitative and qualitative determination of human papillomavirus, HPV, in a sample. More precisely, for quantitative and qualitative determination of oncogenic HPV to predict the risk of HPV infection resulting in cervical carcinoma. The method and kit enable simultaneous measurement of several oncogenic HPV types.